Cultivation of "Difficult" Viruses from Patients with Common Colds

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Viruses can often be isolated from patients with common colds by inoculating their nasal secretions into tissue cultures, but more viruses can be propagated by inoculating such secretions into organ cultures of human embryo nasal or tracheal epithelium (Tyrrell and Bynoe, 1965; Hoorn and Tyrrell, 1966). Many of the viruses which grow in organ cultures can be recognized in the laboratory. We have made preliminary reports (Tyrrell and Bynoe, 1966; Tyrrell, 1967) of studies on a collection of nasal washings. We now wish to make a full report, and in this paper we describe the cultivation of still more viruses by further experiments in which a modified technique of organ culture was used.

Methods and Material

The *volunteers* were aged between 18 and 50 years and were of both sexes. They were cared for and examined as described elsewhere (Tyrrell, 1963). They received 1 ml. of washings or organ culture fluid as intranasal drops which were usually diluted 1:10 in saline. Paired sera and nasal washings were collected from a proportion of those who developed colds.

The *patients* were mainly adults with colds and similar respiratory illnesses who belonged to the laboratory staff or who were visiting the unit as volunteers; but all the patients had apparently contracted upper respiratory infections outside the unit, the volunteers before arrival. The illnesses were usually graded as typical common colds and were usually afebrile. A few children aged 11 to 13 were tested; one of these had a severe pharyngitis with meningism and was found to be infected with adenovirus type 3. Nasal washings were collected within four days of onset and paired sera were sometimes obtained also.

Organ cultures were prepared and handled by the basic technique described by Hoorn (1966), with modifications referred to below. The tissue was obtained from human embryos removed at hysterotomy at the 14th to 24th weeks of gestation. Nasal and tracheal cultures were used almost interchangeably, but in later experiments with "difficult" viruses both nasal and tracheal cultures were inoculated at each passage and pooled fluids were used. For virus isolation attempts 0.2 ml. of nasal washings in 50% broth-saline were inoculated into cultures. Organ culture fluids were mixed with an equal volume of bacteriological nutrient broth and held at about −65° C. until needed. During these studies 116 volunteers were given fluids from uninoculated organ cultures and three developed colds; 120 were given saline and none developed colds.

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Tissue cultures were prepared and handled basically as described by the Medical Research Council Working Party (1965). When viruses are said to be "tested in tissue culture" this means that they were inoculated into cultures of monkey kidney, human diploid, HeLa, and often human embryo kidney cells, and examined in order to detect upper respiratory viruses, including influenza and parainfluenza viruses, adenoviruses, rhinoviruses, and enteroviruses.

Propagation of Previously Uncultivated Viruses

We reported earlier that certain viruses apparently present in nasal secretions could be propagated in organ cultures while others could not (Tyrrell and Bynoe, 1966). Since then we have investigated further the growth of influenza and rhinoviruses in organ cultures (Tyrrell and Blamire, 1967) and have modified and improved our previous technique, the O method, in which we used medium 199 and an atmosphere of air. The main differences are outlined in Table I. We have used the modified (M) technique in attempts to propagate viruses which were present in nasal washings, but which we had failed to grow before. Other features, such as the technique of preparing the tissue and the incubation temperature of 33° C., were unchanged. The cultures were observed for ciliary activity for up to 10 days.

Some of the results summarized earlier and others obtained recently are presented in Table II, which shows that viruses which could not previously be propagated can now be grown; further, the proportion of colds produced suggests that with the improved methods they are growing as freely as those others cultivated by the "original" method. It should be noted that the strain H.G.P. 26/7/57, is not the prototype rhinovirus 2; it is a virus which was previously reported to be neither an M nor an H rhinovirus and to be unable to grow in tissue cultures (Tyrrell and Bynoe, 1961). The strain F.E.B. 22/12/64 is likewise distinct from the prototype rhinovirus strain with the same initials, and represents a washing collected from the same subject when suffering from a later cold.

The cultivation of some of these more "difficult" viruses was performed in a series of experiments which may now be summarized.

Cultivation of Some "Difficult" Viruses

S.T. 28/3/65 (Fig. 2)

This virus was originally passed in O cultures at first in Salisbury and then in Lund. It soon produced obvious ciliary destruction and continued to grow and to produce colds when

TABLE I.—*Technical Details of "Original" and "Modified" Methods of Organ Culture*

		No. of Fragments	Volume of Medium	Medium	Concentration of			Frequency of Changing Medium
					Bicarbonate (g./100 ml.)	CO_2	Bovine Plasma Albumin (g./100 ml.)	
Original or O	Tracheal	5 or 6	1·25	199	0·035	Ambient	0	Daily
	Nasal	2 „ 3	2·5–3·5					
Modified or M	Tracheal	2	1·25	Eagle's (Gibco)	0·1	5%	0·2	4 days
	Nasal	1	2·5–3·5					

inoculated into volunteers after serial passages in M cultures. Even after the final passage it had no effect when inoculated into tissue cultures, but, using arrest of cilia as an index of the presence of virus, we showed it to be ether-stable, acid-labile and to pass a filter of A.P.D. about 50 mμ. It is therefore presumably a fastidious rhinovirus like the H.S. virus previously reported (Hoorn and Tyrrell, 1966). However, it was not neutralized by antiserum against H.S. virus.

M.R.

This agent has been under study at the unit since 1956, when a volunteer, M.R., was given material from an infected tracheal culture and developed a cold three days later. As the series of experiments was otherwise negative it is thought likely that she was infected outside the unit, particularly as the incubation period of colds due to this agent has proved to be unusually long. This is shown in Table III, from which it will be seen that the colds were of long duration. Table III also shows that colds due to avian-infectious bronchitis-like viruses (A.I.B.-like viruses) have a long incubation period, but in these the duration was short. Colds due to M.R. showed more fever and malaise, were graded as severe more often than were colds due to rhinoviruses, and were much less likely to be followed by a cough.

In a series of experiments with the virus some years ago it was shown that volunteers given a second inoculation of wash-

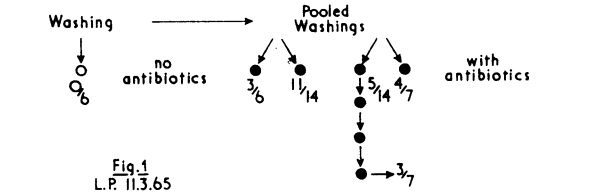
TABLE II

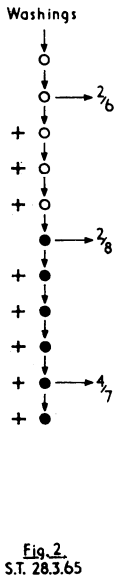
Donor*	Date	Inoculum Patients Washing (P) or Pool of Washings from Infected Volunteers (V)	Results of Inoculation of Fluid from Organ Cultures Maintained by "Original" Method into		"Modified" Method into	
			Volunteers	Tissue Culture	Volunteers	Tissue Culture
H.G.P.	26/7/57	V	1/6†	Rhinovirus		
J.E.D.	24/4/64	P	2/6	,,		
F.T.	30/3/64	P	4/10	,,		
M.T.	/12/62	P	4/15	,,		
B.R.	31/12/63	P	—	,,		
P.D.B.	4/5/65	P	—	,,		
P.K.B.	1/6/65	P	—	,,		
D.T.	11/6/65	P	—	,,		
J.E.D.	12/8/61	P	—	,,		
B814		V		None		
H.W.	20/1/64	V	4/6	Para-influenza		
F.A.L.	26/2/65	P	3/6	,,		
D.T.	2/4/65	P	1/4	,,		
E.V.S.	2/3/65	P	4/6	None		
D.L.J.	2/1/64	P	1/7	None	—	Rhinovirus
M.R.		V	0/12	,,	6/13	None
L.P.	11/3/65	P and V	0/6	,,	3/6	,,
E.W.G.	26/4/65	P	0/6	,,	2/9	Rhinovirus
D.T-R.	16/10/60	P	0/6	,,		
S.T.	28/3/65	P	2/6	,,	4/7	None
F.E.B.	22/12/64	P	3/5	,,	1/8	,,
D.T-R.	19/12/60	P	0/6	,,	1/5	,,
A.	3/3/65	P	0/6	,,		
D.	2/1/64	P	1/7	,,	0/8	,,

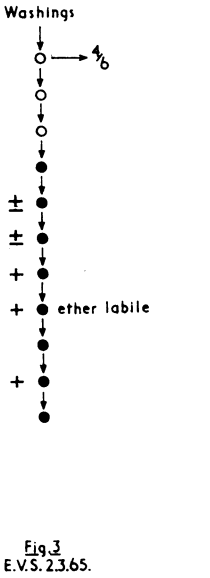
* In addition 9 specimens yielded viruses on direct inoculation into tissue cultures—for example, 2 strains of haemadsorbing virus, 1 of adenovirus, 2 enteroviruses, and 4 rhinoviruses.

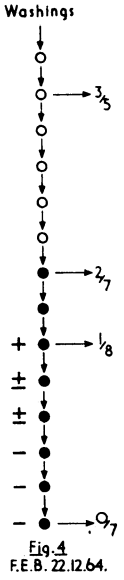
† In these and subsequent tables the numerator denotes the number of colds observed and the denominator the number of volunteers inoculated.

Studies on strain B814, F.T., and M.T. were reported in more detail previously (Tyrrell and Bynoe, 1965).









FIGS. 1-4.—The diagrams summarize the experiments with four agents. Each passage is indicated by an arrow; organ cultures are shown by circles, open if the O technique was used and filled if the M technique. Reduction in ciliary activity is shown as + and doubtful reduction as ±. The fractions indicate the outcome of a volunteer experiment—the numerator the number of colds observed and the denominator the number of volunteers infected. Some test results and experimental conditions are also shown.

TABLE III.—*Clinical Features of Colds Produced by Four Unidentified Viruses Compared with those of Rhinovirus Type 2, and Avian-Infectious Bronchitis-like Viruses*

	M.R.	L.P.	E.V.S.	F.E.B. 22/12/64	Rhinovirus Type 2	A.I.B.-like 229E and B814
No. of volunteers inoculated	207	68	15	27	213	153
No. getting colds (No.)	61	37	11	6	78	78
No. getting colds (%)	29	54	73	22	37	51
Incubation period in days (Mean)	3·8	2·7	3·5	2·8	2·1	3·0
Incubation period in days (Range)	2–6	2–4	3–4	3–4	1–5	2–5
Duration of colds in days (Mean)	10	6	5	7	9	7
Duration of colds in days (Range)	3–18	3–11	2–12	2–19	3–19	2–19
Maximum No. of handkerchiefs used daily (Mean)	17	20	8	12	14	20
Maximum No. of handkerchiefs used daily (Range)	4–60	6–60	5–14	4–32	3–38	4–120
Malaise (%)	49	73	36	66	28	55
Headache (%)	47	65	64	66	56	67
Chill (%)	21	13	27	33	28	22
Pyrexia (%)	29	27	36	16	22	19
Pyrexia (Range)	99·2–101·8°	99·2–100·4°	99·2–100·6°	99·2–100·6°	99·2–100·4°	99·2–103
Mucopurulent nasal discharge (%)	70	27	27	33	83	54
Sore throat (%)	69	35	64	16	87	64
Cough (%)	26	19	18	0	68	54
Severity of colds (Mild)	44–72%	25–68%	9–82%	5–84%	63–81%	52–67%
Severity of colds (Moderate)	8–13%	9–24%	1–9%	1–16%	12–15%	17–22%
Severity of colds (Severe)	9–15%	3–8%	1–9%	—	3–4%	9–11%
Colds in controls (No.)	1/137	1/51	1/14	0/25	2/88	2/103
Colds in controls (%)	0·7	2	7	0	2·3	1·9
Hanks's saline	0/72	0/20	0/6	0/13	2/88	2/67
Uninoculated organ culture fluids	1/65	1/31	1/8	0/12	—	0/36

ings three to nine months later suffered fewer colds than those given infectious material for the first time (Table IV). The virus was passed serially four times in volunteers over 11 years, but there was no evidence of any change in its clinical effects. Washings from volunteers were used for other experiments. For example, as shown in Table V, it was found that the organism was ether-labile and therefore presumably not a rhinovirus, and was not inhibited by tetracyclines and therefore probably not a mycoplasma. In numerous experiments, some of which are also shown in Table VI, we failed to grow it in tissue cultures of the types used to isolate rhinoviruses and other respiratory viruses or in the "original" or "modified" organ cultures mentioned above. However, the organ cultures were further modified by the addition of hydrocortisone acetate to the medium, and then there was evidence that a cold-producing agent was multiplying; after three serial passages in cultures containing 10 μg./ml. of hydrocortisone acetate the fluids produced colds in three out of eight volunteers.

TABLE IV.—*Results of Reinoculating Volunteers with M.R. Agent*

	Frequency of Colds
First inoculation of washing	15/27
Second „ „ „	2/13
First inoculation of volunteers who took part in the same trials as those in which the above second inoculations were made	15/35

TABLE V.—*Some Properties of M.R. Agent*

Inoculum and Experimental Procedure	Frequency of Colds
Washings to volunteers treated with 600 mg. daily demethylchlortetracycline	4/5
Similar inoculum into volunteers given placebo	2/5
Hanks's saline	0/5
Washings held overnight at 4° C. with 20% ethyl ether	1/14
„ „ „ „ 4° C.	8/11
Medium from tissue cultures of human embryo lung fibroblasts WI-26 and WI-38 maintained at 33° C. for 5 days after inoculation with washings	0/11
Medium from organ cultures using:	
(*a*) Medium 199 in air and harvesting daily	0/5
(*b*) „ 199 „ „ with 5% CO_2	0/7
(*c*) „ 199 and 0·2% bovine plasma albumin and 5% CO_2	0/6

TABLE VI.—*Attempts to Propagate M.R. Agent in Modified Organ Cultures Containing Hydrocortisone*

Experiment	Inoculum	Frequency of Colds in Volunteers given Fluids from Cultures Maintained with Indicated Concentration of Hydrocortisone Acetate		
		10 μg./ml.	1 μg./ml.	Nil
75	Nasal washings		6/14	
76	Fluids from expt. 75	2/7	0/7	
98	Nasal washings	4/9	—	3/8
			3/9	
77	„ „	0/7	0/7	0/6
80	„ „	0/8		

In one further experiment it seemed that the organism could grow in the absence of hydrocortisone, while in two others it did not grow at all. It was therefore concluded that although the organism could certainly be cultivated in "modified" organ cultures, hydrocortisone was not necessary; possibly not all batches of culture were suitable. Only doubtful reduction in ciliary activity was observed in infected cultures. Since the organism is ether-labile it is presumably not a rhinovirus, and since it multiplies in the presence of tetracycline it is presumably not a mycoplasma or a psittacosis-like organism.

L.P. (Fig. 1)

The agent in this washing was not successfully passed in original (O) organ cultures, but produced three colds when given to six volunteers. Washings from these volunteers were inoculated into modified (M) cultures, in which it grew in each of four experiments with or without antibiotics and was propagated serially four times. The fluids which produced colds in three out of seven volunteers had no effect in tissue cultures. There is therefore no doubt that this virus can now be grown, but whether because a new inoculum was used or because the medium was changed it is impossible to be sure. Washings were held overnight at 4° C. with 20% ethyl ether. None of seven volunteers given this material developed colds, whereas colds occurred in four out of seven given material held overnight at 4° C. without ether. The virus is presumably ether-labile and not a rhinovirus.

E.V.S. 2/3/65

This virus did not prove difficult to propagate in organ cultures, but has been difficult to identify. As can be seen from Fig. 3, after serial passage the organism reduced ciliary activity in infected cultures, and with this as a criterion of the presence of infectious virus it is shown to be ether-labile. The original washings produced colds in seven out of nine volunteers, but after treatment with ether the material failed to produce colds in eight volunteers. No virus was isolated in tissue culture from fluids for the eighth organ-culture passage. The symptoms produced by L.P., E.V.S., and F.E.B. 22/12/64 were not thoroughly evaluated owing to the small number of colds available for study. The clinical picture, however, showed deviations from those produced by rhinoviruses, which were generally in the same direction as those seen with M.R., except that the illnesses were rather short in duration, with a low frequency of mucopurulent nasal discharge and a high frequency of headache.

F.E.B. 22/12/64 and D.T-R. 19/12/60

The studies on these specimens remain inconclusive. Fig. 4 shows that serial passage of the F.E.B. agent was apparently successful in O and M organ cultures, but the fluids produced fewer colds as time went on and inhibition of ciliary activity was inconstant. The organism may have been lost or have become attenuated for man, but it certainly grew at first. The results were similar with D.T-R. 19/12/60, which apparently reduced ciliary activity on first passage while the culture fluids produced colds in one out of five volunteers, but material from later passages did not cause colds in volunteers.

Classification of Viruses Cultivated

The top of Table II lists viruses which were adapted from organ culture to tissue culture and have been shown to be rhinoviruses. Two, F.T. and M.T., have been mentioned in a previous publication (Tyrrell and Bynoe, 1965). In most cases a typical cytopathic effect was observed after inoculating second-passage organ-culture fluid into sensitive human embryo fibroblast cells. Occasionally the ability to affect fibroblasts was acquired more slowly. Some viruses could be propagated only with difficulty in fibroblast cultures, but this was possible with all but one (S.T.), and they have also been grown in M HeLa cells in the presence of additional magnesium (Fiala and Kenny, 1966; Stott and Tyrrell, 1968). The titres obtained were often low, about $10^{2.5}$ TCD_{50}/ml., so it was not always possible to show the conventional hundredfold decline in titres in tests of acid stability. All the viruses listed, however, have been shown to produce a cytopathic effect resembling that of picornaviruses, and to be ether-stable acid-labile, and to pass a collodion filter of A.P.D. about 50 mμ. Many of them have been tested with the available rhinovirus antisera and some apparently belong to known serotypes. A detailed account of this work is in preparation.

After serial passage in organ cultures two viruses multiplied and caused haemadsorption in tissue cultures of rhesus monkey kidney cells. These strains, H.W. and F.A.L., are now believed

TABLE VII.—*Results of Attempts to Isolate and Classify Viruses*

	Entero	Rhino	Parainfluenza	Adeno
Viruses isolated and studied in tissue culture	C.J.B. 12/4/65 (Coxsackie A21) N.J.D. 29/4/65 (? Echovirus untyped)	E.M.B. 10/11/60 N.J.D. 24/4/64 G.T. 16/12/63 M.McM. 29/12/61	F.E.B. 10/12/64 D.T. 2/4/65	S.C.T. 24/7/65 (Type 3)
	A.I.B.-like	Rhino	Parainfluenza	Unidentified Ether-labile
Viruses cultivated in organ culture and grown and studied in tissue culture or organ culture	B814	H.G.P. 26/7/57 J.E.D. 12/8/61 F.T. 30/3/64 M.T. 00/12/62 B.R. 31/12/63 J.E.D. 24/4/64 P.D.B. 4/5/65 P.K.B. 1/6/65 D.L.J. 21/1/64 E.W.G. 26/4/65 D.T. 11/6/65 S.T. 28/3/65 (grows only in organ culture)	F.A.L. 26/2/65 H.W. 20/1/64	M.R. E.V.S. 2/3/65 L.P. 11/3/65
Partially successful cultivation and no properties determined	F.E.B. 22/12/64 D.T-R. 19/12/60			
No virus in specimen and no virus grown	A. 3/3/65 D. 2/1/64			

We have excluded from this Table the specimen D.T-R. 16/10, from which no virus was grown, but which could not be tested in volunteers or in modified cultures, and B, from which a virus was apparently grown in tissue culture but the specimen was exhausted and the virus was lost on passage.

to be parainfluenza viruses, and a detailed account of serological and clinical studies with these organisms will also be published. The first two of these strains were originally suspected to be paramyxoviruses because the virus particles were visualized with the electronmicroscope (Tyrrell and Almeida, 1968). For completeness we should also mention here the strain B814, which was shown to be an avian-infectious-bronchitis-like virus by electronmicroscopy. However, the ether-labile viruses, M.R., L.P., and E.V.S., could not be identified either by tissue culture or by electronmicroscopy. The remaining agents were propagated with such difficulty that it has not been possible to study their properties.

We have summarized the results of virus isolation and classification so far in Table VII. This shows that a definite or presumed virus was grown from 29 out of 31 specimens and that the remaining two did not produce colds in volunteers. One and possibly two viruses could not be propagated serially for certain. Fourteen viruses, 11 of them rhinoviruses, which could not be detected in tissue culture, were grown in organ cultures and detected and classified either by adapting them to tissue cultures or in one instance by electronmicroscopy and in another by observing reduction in the ciliary activity of organ cultures.

Three further viruses were certainly grown in organ cultures; they appear to be ether-labile, but have not been shown to be any of the known ether-labile viruses of the respiratory tract.

Discussion

It is clear that the techniques used were successful in propagating viruses from colds which had occurred over a long period of time, and included some which had been tested repeatedly by earlier methods without success. Some of the experiments with M.R. are mentioned here and, with B814, have been described before (Tyrrell and Bynoe, 1965), but the strain H.W. and some others were also repeatedly tested.

Relatively few patients have been studied, but we believe we obtained a representative sample of the colds of adults in our area. If anything, the sample is somewhat loaded in favour of "difficult" viruses, since it includes strains, like H.G.P., M.R., H.W., and B814, which were included because after intensive study they had failed to grow in tissue cultures. On the other hand, we did take pains to collect nasal washings from quite definite and typical cases. Our rate of isolating rhinoviruses in tissue culture is lower than that in an earlier study from this laboratory (Kendall *et al.*, 1962) and elsewhere (Hamre *et al.*, 1966), but the numbers are rather small and so the difference is not statistically significant. Phillips *et al.* (1965) recovered rhinoviruses from about a quarter to a half of three series of students with colds by the use of a number of different strains of cells. Some of our specimens containing rhinoviruses—for example, H.G.P. 26/7/57—had been tested in human embryo kidney and several different strains of fibroblasts, and failed to produce a recognizable cytopathic effect. It therefore seems to us unlikely that the apparent improvement in the isolation of rhinoviruses when organ cultures were used was an artifact due to inefficient testing in tissue cultures. The improvement has in fact already been confirmed independently (Higgins, 1966).

The aetiology and epidemiology of colds do not vary greatly in most centres of population, and it is therefore likely that it will be found elsewhere that almost all common cold viruses can be grown in organ cultures of human ciliated epithelium and that most viruses can be identified by the combination of organ culture with other techniques. On the other hand, it is probable that the distribution of viruses between the different groups and serotypes will be found to vary considerably from time to time and from place to place. The unidentified ether-labile viruses should be studied further.

Though we still await further studies on the serotyping of viruses, it is clear already that the use of organ cultures has not greatly changed the aetiological pattern revealed by the use of tissue cultures. Thus rhinoviruses are still the predominant organisms recovered, they belong to various serotypes, and, just as some rhinoviruses may be recovered in organ culture but not directly in tissue culture, so some parainfluenza viruses were detected in organ cultures and not in tissue cultures. However, organ cultures are apparently needed to grow the A.I.B.-like viruses.

Summary

Specimens were collected during 31 colds in 27 patients, mainly adults. The specimens were tested for the presence of viruses by the combined use of tissue culture, organ culture, and inoculation of volunteers. Known or presumed viruses were grown from 29 specimens. These included 16 rhinoviruses, 2 enteroviruses, 4 parainfluenza viruses, 1 virus resembling avian infectious bronchitis virus, 1 adenovirus, and 5 unclassified agents, probably viruses. Of these, 4 rhinoviruses, 2 enteroviruses, 2 parainfluenza viruses, and 1 adenovirus were detected by the use of ordinary tissue cultures only, organ cultures being unnecessary.

We wish to thank the volunteers for their willing co-operation and Dr. H. E. M. Kay and his staff for the supply of most of the embryos used. Miss E. M. Bullock assisted in the volunteer

experiments, and Miss C. J. Blamire and Mrs. P. K. Brown in the laboratory work. Dr. P. J. Chapple carried out some of the preliminary experiments on strain H.W., and Drs. H. G. Pereira and A. T. Roden on strain M.R.

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Necrotic Cervicitis Due to Primary Infection with the Virus of Herpes Simplex

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[WITH SPECIAL PLATE FACING PAGE 602]

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Since July 1965 I have encountered in London six cases of extremely severe and necrotic cervicitis, the whitish appearances of which were strikingly similar. The condition in the first case was unfamiliar, but with subsequent cases evidence has been assembled which strongly suggests that the cervicitis is due to the virus of herpes simplex.

Present Series

Case Material.—The basic data concerning the six cases are given in Table I. The women were aged 20–28 years (average 23.3 years), four were white and two Negro, and three were single and three married. All were attending the venereal diseases clinic for the first time and none gave a history of any previous venereal infection.

TABLE I.—*Basic Data*

Case No.	Age in Years	Race	Married or Single	Occupation	Previous History of Venereal Disease
1	21	White	S	Receptionist	No
2	20	White	S	Sales promotion	No
3	23	Negro	M	Canteen assistant	No
4	21	Negro	S	Machinist	No
5	28	White	M	Housewife	No
6	27	White	M	Public relations	No

Symptoms.—The presenting symptoms are given in Table II. All six patients complained of vaginal discharge, four of dysuria, three of local soreness, and four of pain in the lower abdomen, right iliac fossa, or the genitals. In Case 1 the dysuria was severe, and twice daily catheterization had been performed in Spain before the patient decided to fly home to take further advice. The onset was acute or subacute, symptoms having been present for from one to nine days before they attended the clinic.

Findings on Examination.—These are shown in Table III and in the Special Plate. In all cases there was gross cervicitis, from which pieces of dead tissue might separate, leaving a white necrotic core around the os. Pain on touching or moving the cervix was elicited in four cases, and in all six the cervix bled easily. The inguinal lymph nodes were enlarged and tender in only one case. There was some known fever in four cases, but in all except one this was not severe at the time of recording, though it may well have subsided by the time the patients were first seen. In one case it was known to have reached 104° F. (40° C.). The presumptive or alternative diagnosis on these cases at the first cervical inspection ranged from gonorrhoea (two cases), salpingitis (two cases), carcinoma of the cervix, leucoplakia, and the effects of cautery.

TABLE II.—*Symptoms*

Case No.	Vaginal Discharge	Dysuria	Local Soreness	Pain	Duration of Symptoms
1	Yes	Yes*	Yes	Yes, lower abdomen	10 days†
2	,,	,,	,,	,, R.I.F.	2 ,,
3	,,	,,	,,	,, ,,	9 ,,
4	,,	,,	No	,, genital	3 ,,
5	,,	No	,,	No	4 ,,
6	,,	,,	,,	,,	1 ,,

* Required repeated catheterization. † Had been treated elsewhere after only a few days of discharge and dysuria.

TABLE III.—*Findings on Examination*

Case No.	Cervicitis	Pain on Touching or Moving Cervix	Tender Inguinal Nodes	Fever (° F.)	Presumptive or Alternative Diagnosis at First Clinical Examination
1	+ +	Yes	No	99°, ? earlier more so	Gonorrhoea, salpingitis, effects of cautery
2	+ +	,,	Yes	99°	Salpingitis
3	+ +	,,	No	No	Carcinoma of cervix
4	+ +	,,	,,	—	—
5	+ +	No	,,	98·6°, earlier probably more so	Leucoplakia of cervix
6	+ +	,,	,,	104°	Gonorrhoea

Investigations

The results of some of the investigations made on these patients are shown in Table IV. The tests for venereal disease, which included urethral and cervical smears and cultures for gonococci, an examination of a wet specimen of vaginal exudate for trichomonads, a dark-field examination of a cervical speci-

* St. Mary's Hospital, London W.2.